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REGIONAL SURVEY OF GENE FREQUENCIES IN THE MUD SNAIL *NASSARIUS OBSOLETUS*

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One of the most abundant species of the littoral and estuarine environments of the Atlantic coast of the United States is the mud snail, *Nassarius obsoletus*. Adult snails are deposit feeders and scavengers occurring in large numbers on organic-rich intertidal flats from the Gulf of St. Lawrence in Canada to northern Florida (Scheltema, 1964). *Nassarius obsoletus* is an active species with a keen olfactory sense (Carr, 1967; Schaefer, 1969) and a tendency toward aggregative and schooling behavior (Jenner, 1956, 1957, 1958, 1959; Crisp, 1969). Dispersal is primarily by means of a planktonic larva (Scheltema, 1961, 1962).

Recently techniques combining zone electrophoresis and staining for specific proteins that are distinguishable as the products of individual gene loci have begun to contribute to the understanding of genetic systems of marine invertebrates (Manwell and Baker, 1970; Milkman and Beaty, 1970; Selander, Yang, Lewontin, and Johnson, 1970; Gooch and Schopf, 1970, 1971; Schopf and Gooch, 1971).

Electrophoresis and chromatography have had prior application in the genus *Nassarius*. Interspecific differences occur in protein patterns of chromatograms of British *Nassarius* (Collyer, 1961). Isozyme patterns of malate dehydrogenase in adult snails were studied by Meizel and Markert (1967), and electrophoretic activities of several enzymes in embryos and larvae were reported by Goldberg and Cather (1963) and Morrill and Norris (1965).

We report here the results of a study of the electrophoresis genetics of *N. obsoletus* which was begun with a twofold goal: (1) to discover and characterize gene loci from the pattern of their protein products on gels, and (2) to survey populations along a long coastal transect for geographic variation in the sampled portion of the genome.

Materials and Methods

Eleven collections of *N. obsoletus*, of 30 to 184 snails each, were made from January, 1969 to August, 1970 along a 1000 km Atlantic coast transect (Fig. 1). The transect ranges from Cape Cod Bay, Massachusetts, to near Beaufort, North Carolina, and embraces part or all of 3 zoogeographic provinces, the Acadian (1 collection), the Virginian (8), and the Carolinian (2).

Sampled populations represent a diversity of habitats: the sandy sublittoral (Cape Henlopen, Delaware), a salt marsh tidal creek (Canary Creek Marsh,

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Delaware), an estuary (Atlantic City, New Jersey), and intermittently exposed sand and mud flats (Barnstable Harbor and Eel Pond, Massachusetts; Southport, Old Mill Beach, and Stamford, Connecticut; Slaughter Beach, Delaware; and Pivers Island and Shell Point near Beaufort, North Carolina).

Snails were taken randomly by size and sex at most localities. Sites collected by the authors (all but Barnstable Harbor, Eel Pond, and Atlantic City) were sampled by taking all snails in the upper 2–3 cm of sediment of an area 1 m^2. Individuals were maintained alive and without food for a week or more to empty the digestive tract, since ingested matter may cause gel artifacts. They were then electrophoresed immediately or frozen at −60° C for later electrophoresis.

For each electrophoretic run snails were measured for apex to aperture height (Scheltema, 1964) and, when possible, were sexed (penis = male, nidamental gland = female). Sexing of some individuals proved very difficult because the copulatory apparatus of non-breeding snails is often inconspicuous (Jenner and Chamberlain, 1955), and genetic comparisons by sex are not presented below.

From each snail approximately 0.1 g of foot muscle was macerated with a ground glass rod in a 1 ml polystyrene centrifuge tube (Thomas Co., Philadelphia) in 100 μl cold 0.1 M Tris-glycine buffer, pH 8.5, and 25 per cent sucrose solution. Other tissues gave the same qualitative results when prepared identically. Tubes were centrifuged 20 minutes at 7000 rpm. Aliquots of 15 μl of supernatant were pipetted into slots in 6 per cent polyacrylamide gel (apparatus of E. C. Corp., Philadelphia) and gels were run vertically at 75–125 mA, 400–450 v at about 5° C for 2½ hours. Following electrophoresis gels were stained and bands were drawn or photographed for future reference.

Malate dehydrogenase (MDH) and lactate dehydrogenase (LDH)

Electrophoresis buffer was Tris-glycine, pH 8.5, as above. Gels were stained in 0.04 M Tris-HCl buffer, pH 7.2, with a staining mixture of 30 mg NAD, 40 mg nitro BT chloride, and 3 mg phenazine methosulfate per 100 ml of solution. Substrates were the sodium salts of malic and lactic acid (Sigma Co., St. Louis). Bands of NAD-dependent MDH developed within 2 hours in the dark at room temperature, but the much lower assay of LDH required overnight staining.

General protein

Gel proteins were fixed in an aqueous solution of 12 per cent trichloroacetic acid and then stained 6–8 hours in a solution of 12 per cent trichloroacetic acid with 6 ml 1 per cent aqueous Coomassie Blue (Colab Co., Chicago) added per 100 ml.

Tetrazolium oxidase

Achromatic zones, operationally termed tetrazolium oxidase (Baur and Schorr, 1969), were visualized with the MDH and LDH staining systems with substrate omitted and staining in full light for 2–3 hours.

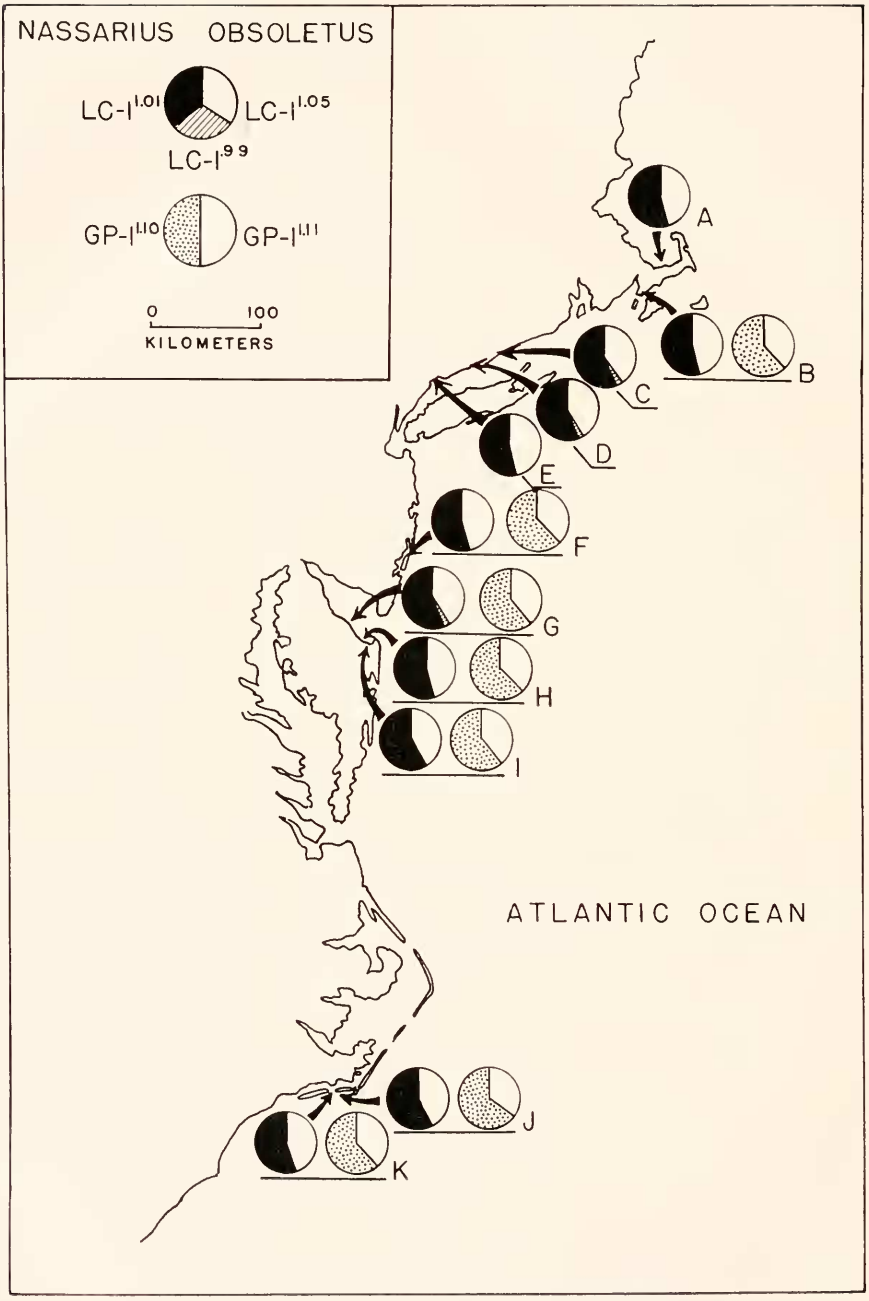

FIGURE 1. The transect of *Nassarius obsoletus* from Cape Cod, Massachusetts, to Beaufort, North Carolina. Allele frequencies at the Lc-1 locus are indicated as percentages of the shoreward circles and those of the Gp-1 locus by the seaward circles. Localities are:

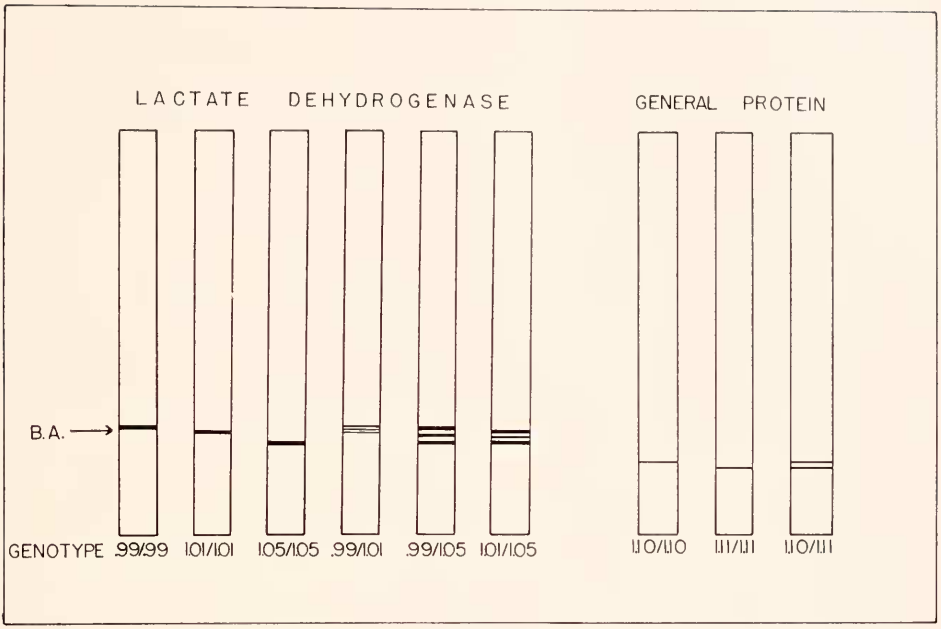

FIGURE 2. Diagram indicating band patterns and mobility relationships of protein zones belonging to the Lc-1 and Gp-1 loci. Homozygotes are single-banded and heterozygotes are three-banded (Lc-1 locus) and two-banded (Gp-1 locus). Arrow marks the mobility of bovine serum albumin under standard electrophoresis conditions.

Leucine aminopeptidase (LAP)

Electrophoresis buffer was 0.09 M Tris-borate, pH 8.8. Staining procedure was identical to that of Gooch and Schopf (1970) for LAP in ectoprocts.

Bovine serum albumin (Nutritional Biochemicals Corp.) was electrophoresed on each gel and stained with Coomassie Blue to serve as a reference standard for band mobility.

Other enzyme systems are under analysis. Esterase, glucose-6-phosphate dehydrogenase, and phosphohexose isomerase loci have been defined, but not studied along the transect. No activity was detected for ribonuclease, acid phosphatase, alpha-glycerophosphate dehydrogenase, and isocitrate dehydrogenase.

RESULTS

Gene loci

Six gene loci, 4 monomorphic (67 per cent) and 2 polymorphic (33 per cent), were characterized from gel patterns of the 5 protein systems (Table I). As with

A, Barnstable Harbor, Massachusetts; B, Eel Pond, Massachusetts; C, Southport, Connecticut; D, Old Mill Beach, Connecticut; E, Stamford, Connecticut; F, Atlantic City, New Jersey; G, Slaughter Beach, Delaware; H, Canary Creek, Delaware; I, Cape Henlopen, Delaware; J, Shell Point, North Carolina; K, Pivers Island, North Carolina.

other organisms difficult or impossible to cross under controlled conditions, the interpretation of genotypes is based on electrophoresis patterns. See Selander, Yang, Lewontin, and Johnson (1970) and Gooch and Schopf (1970) for the detailed rationale. In brief, monomorphism is indicated for a locus where all individuals yield bands of identical mobility, and polymorphism is indicated where bands of 2 or more mobility classes occur, both singly and in all heterozygous combinations (Fig. 2).

Malate dehydrogenase. NAD-dependent malate dehydrogenase is the product of the locus M-1 with a single allele, $M\text{-}1^{.29}$, present in all populations (Table I). Superscripts of alleles refer to mobility of bands against the bovine albumin standard; nomenclature is discussed by Gooch and Schopf (1970). Under other electrophoretic conditions MDH may appear as 3 or more isozyme bands of variable mobility (Goldberg and Cather, 1963; Meizel and Markert, 1967), but the number of isozymes is apparently not genetic (Meizel and Markert, 1967).

TABLE I

Enzyme systems and defined gene loci in N. obsoletus

Enzyme system	Locus	Number and designation of alleles
General protein	Gp-1	2 $Gp\text{-}1^{1.10}$, $Gp\text{-}1^{1.11}$
Malate dehydrogenase	M-1	1 $M\text{-}1^{.29}$
Lactate dehydrogenase	Lc-1	3 $Lc\text{-}1^{.99}$, $Lc\text{-}1^{1.01}$, $Lc\text{-}1^{1.05}$
Tetrazolium oxidase	To-1	1 $To\text{-}1^{.44}$
	To-2	1 $To\text{-}2^{.71}$
"Leucine" aminopeptidase	Lap-1	1 $Lap\text{-}1^{.70}$

About 1 to 2 per cent of individuals of most populations show a broad, poorly defined band of MDH activity of the usual mobility. We cannot exclude the possibility of the diffuse zone representing a genetic variant. It will be treated operationally as identical to the allele characterized by the sharply defined band.

Lactate dehydrogenase. LDH is the product of the autosomal locus, Lc-1, with 3 codominant alleles, $Lc\text{-}1^{.99}$, $Lc\text{-}1^{1.01}$, and $Lc\text{-}1^{1.05}$ (Table I, Fig. 2). The presence of a hybrid band of intermediate mobility in heterozygotes denotes that LDH polypeptides probably associate as dimers.

General protein. Up to 8 faint and poorly defined band systems occur on gels stained for general protein. Protein bands of the locus Gp-1 are sharply resolved and permit the identification of 2 apparently autosomal and codominant alleles, $Gp\text{-}1^{1.10}$ and $Gp\text{-}1^{1.11}$ (Table I, Fig. 2). Heterozygotes are double-banded, indicating that the protein is probably not multimeric.

Tetrazolium oxidase. Two band systems occur on photocatalyzed tetrazolium-stained gels. They are operationally regarded as representing separate loci, To-1 and To-2. Both are monomorphic for single alleles, respectively, $To\text{-}1^{.44}$ and $To\text{-}2^{.71}$ (Table I). Three widely spaced populations only were surveyed for tetrazolium oxidase systems, Southport, Slaughter Beach, and Shell Point.

"Leucine" aminopeptidase. Gels stained for LAP possess a single zone of enzyme activity as the product of the locus Lap-1 (Table I). The Barnstable Harbor population was not surveyed for LAP.

Genetics of populations

The loci M-1, To-1, To-2, and Lap-1 are monomorphic for the same alleles in all sampled populations and thus do not contribute to regional genetic variation. The diallelic Gp-1 and triallelic Lc-1 loci segregate for the same alleles along the entire transect. The effects of age structure and geographic distance on allele and genotype frequencies were investigated.

Allele and genotype frequency in relation to age structure. The apex to aperture height increases with age in *N. obsoletus*, although individuals of a given age class may vary several mm (Morrison and Medcof, 1943; Scheltema, 1964). In some populations it is possible to distinguish age classes up to 2 years plus from discrete modal classes in height-frequency plots (Scheltema, 1964).

TABLE II

Snails from Connecticut populations partitioned into size classes. Genetic data for the Lc-1 locus and comparisons of genotype distributions with Hardy-Weinberg values are given for each size class

Size class (mm)	Sample size	Allele frequency		Genotype distribution			Chi-square accord with Hardy-Weinberg equilibrium
		$Lc\text{-}1^{1.01}$	$Lc\text{-}1^{1.05}$	1.01/1.01	1.01/1.05	1.05/1.05	
8–9	32	0.578	0.422	9	19	4	1.5, $p > 0.20$
10–11	65	0.523	0.477	16	36	13	0.8, $p > 0.30$
12–13	78	0.545	0.455	24	37	17	0.1, $p > 0.70$
14–15	95	0.584	0.416	34	43	18	0.4, $p > 0.50$
16–17	68	0.566	0.434	20	37	11	0.9, $p > 0.30$
18–21	30	0.600	0.400	11	14	5	0.02, $p > 0.80$
Pooled	368	0.561	0.439	114	186	68	0.3, $p > 0.50$

If natural selection has strong differential action on age classes, heterogeneity might appear in allele and genotype frequencies of populations of pooled size classes. A 2×6 table test of homogeneity for allele frequency *versus* size class at the Lc-1 locus for 368 snails of pooled Connecticut populations gives no evidence of heterogeneity (Table II; $\chi^2_{(5)} = 1.8$, $P > 0.8$; the rare genotypes bearing the 0.99 allele were not analyzed here or in subsequent comparisons). For feasibility of pooling procedure see next section. Homogenity chi-square for conformance of genotype distributions of size classes to Hardy-Weinberg expectations and pooled conformance also evidence non-significant values (homogeneity $\chi^2_{(5)} = 3.4$, $P > 0.6$; pooled $\chi^2_{(1)} = 0.4$, $P > 0.5$).

Tests of homogeneity of allele frequency with size class were also done for 136 snails from pooled Delaware populations. Nonhomogeneous distributions were not found for either locus (Lc-1 $\chi^2_{(3)} = 1.6$, $P > 0.5$; Gp-1 $\chi^2_{(3)} = 0.9$, $P > 0.8$).

Allele and genotype frequencies along the transect: Lc-1 Locus. Analysis of the Lc-1 locus of 746 individuals discloses the segregation of the 1.01 and 1.05 alleles in all populations of the transect (Table III). The 0.99 allele appeared solely in heterozygotes and in only 3 populations (Table III). Presumably more intensive sampling would reveal its presence in other populations. Overall allele frequencies are $Lc\text{-}1^{.99}$, 0.003; $Lc\text{-}1^{1.01}$, 0.548; and $Lc\text{-}1^{1.05}$, 0.449.

Genotype distributions in all populations agree with Hardy-Weinberg values (Table III). Pooled genotype distribution falls within Hardy-Weinberg limits ($\chi^2_{(1)} = 1.0$, $P > 0.3$; homogeneity $\chi^2_{(9)} = 16.1$, $P > .05$). Allele frequencies are also remarkably uniform (2×11 table homogeneity $\chi^2_{(10)} = 5.5$, $P > 0.8$). Even small inter-population differences in allele frequency should depress the pooled observed frequency of heterozygotes compared with Hardy-Weinberg frequencies based on pooled allele frequencies (Wahlund principle of Wallace, 1968). On the contrary an excess of 3 per cent observed hetrozygotes (non-significant) was found.

TABLE III

Allele frequencies and genotype distributions for polymorphic loci along the transect. Caption of Figure 1 gives the localities listed here by letters. Genetype distributions are compared to Hardy-Weinberg expectations by chi-square tests

Locality	Sample size	Allele frequency			Genotype distribution						Chi-square accord with Hardy-Weinberg equilibrium
Lc-q locus		Lc-$1^{0.99}$	Lc-$1^{1.01}$	Lc-$1^{1.05}$	0.99/ 0.99	0.99/ 1.01	0.99/ 1.05	1.01/ 1.01	1.01/ 1.05	1.05/ 1.05	
A	44	0	0.523	0.477	0	0	0	9	28	7	3.3, $P > 0.05$
B	54	0	0.528	0.472	0	0	0	12	33	9	2.8, $P > 0.05$
C	185	0.003	0.595	0.402	0	1	0	63	93	28	0.4, $P > 0.40$
D	129	0.004	0.531	0.465	0	0	1	37	63	28	0.02, $P > 0.80$
E	58	0	0.517	0.483	0	0	0	14	32	12	0.6, $P > 0.40$
F	30	0	0.517	0.483	0	0	0	10	11	9	2.1, $P > 0.10$
G	77	0.013	0.532	0.455	0	1	1	25	31	19	2.1, $P > 0.10$
H	31	0	0.516	0.484	0	0	0	10	12	9	1.5, $P > 0.20$
I	31	0	0.565	0.435	0	0	0	10	15	6	0.02, $P > 0.80$
J	54	0	0.574	0.426	0	0	0	16	30	8	1.0, $P > 0.30$
K	53	0	0.519	0.481	0	0	0	11	33	9	3.1, $P > 0.05$
Pooled	746	0.003	0.548	0.449	0	2	2	217	381	144	1.0, $P > 0.30$
Gp-1 locus		Gp-$1^{1.10}$	Gp-$1^{1.11}$		1.10/1.10			1.10/1.11		1.11/1.11	
B	52	0.615	0.385		20			24		8	0.02, $P > 0.80$
F	13	0.692	0.308		5			8		0*	2.0, $P > 0.10$
G	71	0.634	0.366		29			32		10	0.1, $P > 0.70$
H	32	0.656	0.344		12			18		2*	1.6, $P > 0.20$
I	32	0.688	0.312		14			16		2*	0.7, $P > 0.30$
J	47	0.713	0.287		21			25		1*	2.8, $P > 0.05$
K	48	0.635	0.354		21			19		8	1.0, $P > 0.30$
Pooled	295	0.654	0.346		122			142		31	0.1, $P > 0.70$

* Homozygote classes pooled.

Allele and genotype frequencies along the transect: Gp-1 Locus. Homogeneity of allele and genotype frequency is also manifest at the Gp-1 locus. In all 295 snails from 7 populations were surveyed. None of the genotype distributions differ significantly from Hardy-Weinberg values (Table III). Pooled genotypes fit Hardy-Weinberg values ($\chi^2_{(1)} = 0.1$, $P > 0.7$; homogeneity $\chi^2_{(5)} = 8.2$, $P > 0.1$). Again there is a non-significant excess of pooled observed heterozygotes, 6 per cent. Allele frequencies appear homogeneous (2×7 table, $\chi^2_{(6)} = 2.9$, $P > 0.8$).

Interaction between loci. Lacking knowledge of the linkage or epistatic relationships of the Lc-1 and Gp-1 loci, it cannot be presumed that their alleles assort independently or maintain random combinations. Linkage disequilibrium or posi-

tive or negative epistatic interactions may affect frequences of allelic combinations Deficiencies in certain gene combinations occurred in two "nothing" dehydrogenase loci in the polychaete *Hyalinoccia tubicola* (Manwell and Baker, 1970).

Genotypes at both loci were determined for 131 individuals from pooled Delaware populations (Table IV). Combination frequencies conform to random expectations ($\chi^2_{(7)} = 7.1$, $P > 0.3$), indicating the absence of detectable interaction between loci.

TABLE IV

Genotype combinations of the Lc-1 and Gp-1 loci in pooled Delaware populations. Figures in parentheses are values expected by random combination

Genotypes			
Gp-1 locus	Lc-1 Locus		
	1.01/1.01	1.01/1.05	1.05/1.05
1.10/1.10	21 (16.2)	23 (27.5)	11 (11.8)
1.10/1.11	15 (17.4)	28 (29.5)	18 (12.6)
1.11/1.11	7 (4.7)	5 (8.0)*	3 (3.4)*

* Pooled for chi-square.

DISCUSSION

The portion of the genome of *N. obsoletus* sampled by electrophoresis genetics presents a uniform picture along the coastal transect. At 4 loci single alleles have become established in all tested populations. At 2 polymorphic loci allele frequencies are virtually constant over the transect. Allele frequencies are homogeneous over age classes. Deviations from Hardy-Weinberg genotype distributions were not detected in 18 tests of individual populations and 2 of pooled populations.

Is the rest of the genome uniform geographically? Adaptive genetic differences along the transect should be manifested by geographically variable developmental and physiological tolerances. Clinal differences in developmental rate relative to temperature occur commonly in Pacific coast gastropods (Dehnel, 1955). There is no evidence for such differences in *N. obsoletus*. Temperature affects the rate of egg development of snails from Cape Cod and Beaufort almost identically (Scheltema, 1967). Reproductive timing in Atlantic Coast populations can be explained as response of the same genotype to environmental induction (Sastry, 1971). Adults from the cold waters of the Cape Cod Canal are remarkably tolerant to elevated water temperatures compared to other species of canal invertebrates examined (populations maintained at 35° C for 42 days before the onset of mortality: Pearce, Silverman, and LeGoff, 1968). The possession of higher thermal tolerance than is locally required is consistent with the hypothesis that the thermal adaptation, presumably genetic, of more southerly populations also exists in the Cape Cod Canal population.

The evidence presented above suggests that minimal genetic differentiation of populations exists along the transect and perhaps throughout the remainder of the

species range. Exploring this hypothesis further, we may ask how such genetic uniformity is maintained. Regional uniformity of allele frequencies may be ascribed to (1) mechanisms of balancing selection, such as heterosis and frequency-dependent selection, or (2) pervasive gene flow opposing local selection and promoting regional panmixia.

Various kinds of balancing mechanisms have been proposed to explain the unexpectedly low variability in allele and karyotype frequencies found in some studies of animals with weak or moderate powers of dispersal (Carson, 1965; Prakash, Lewontin, and Hubby, 1969; Berger, 1971, using species of *Drosophila;* Burns and Johnson, 1971, on the butterfly *Hemiargus isole;* Koehn, Perez, and Merritt, 1971, in conjunction with the freshwater fish *Notropis stramineus*). In animals with greater dispersal abilities gene flow is usually held as the cause of regional stabilization of gene frequencies (Merrell, 1970, in reference to the *burnsi* gene in *Rana pipiens*).

Balancing mechanisms cannot maintain areal uniformity of allele frequencies if the adaptive values of genotypes are dependent on geographically variable elements of the environment. The more heterogeneous the environment the less likelihood there is of balancing selection alone maintaining uniformity. There is considerable environmental hetrogeneity along the coastal transect. The approximate yearly temperature variation at localities along the transect amounts to 0° to 18° C at the entrance of the Cape Cod Canal into Cape Cod Bay (Fairbanks, Collings, and Sides, 1968), 5° to 22° C off Cape Henlopen, Delaware (Cronin, Daiber, and Hulbert, 1962), and 7.9° to 27.6° C in the Beaufort estuaries (Williams, 1967). Temperature gradation along the transect is not entirely clinal. Sharp thermal discontinuities persist throughout much of the year off Cape Cod and Cape Hatteras and delimit the boundaries of zoogeographic provinces (Johnson, 1934; Hedgpeth, 1953; Cerame-Vivas and Gray, 1966). The winter thermal gradient near Cape Hatteras is sometimes a 14° C change over 8 nautical miles (Stefansson, Atkinson, and Bumpus, 1971). Inshore temperature variation is, of course, less drastic. These temperature differences exceed the tolerances of many species, limiting the species range or giving rise to physiological races (Segal, 1964; Vernberg and Vernberg, 1970).

Other aspects of the environment varied between collecting localities, but less systematically. Snails were collected on substrates varying from dark organic muds to coarse sands, and in salinities between 16‰ and 30‰. Undoubtedly variation occurred in current energy, diet, biotic association and other factors, but these were not studied in sufficient depth to evaluate here.

The remarkable physiological toughness of *N. obsoletus* that enables it to flourish in a diversity of environments may be partly due to heterotic mechanisms. However, we find it difficult to conceive of selection coefficients totally unresponsive to the environmental variation encountered by snail populations. For this reason balancing selection alone is not a convincing explanation for regional uniformity of allele frequencies.

Adult snails are capable of limited migratory activity (Batchelder, 1915; Crisp, 1969), but the true disperal stage and agent of gene flow is the planktonic larva. The efficacy of larval dispersal in widely distributed marine animals is well known (Johnson, 1939; Thorson, 1961; Robertson, 1964; Gurjanova, 1968;

Scheltema, 1966, 1968, 1971). Long-lived larvae may be transported transoceanic distances (Scheltema, 1971). In the shallow seas adjacent to continents tidal, rip, seasonal, and permanent currents distribute larvae over extensive areas (Mileikovsky, 1966). Off the Atlantic coast between Cape Cod and the Carolinas surface current drift is predominantly longshore with occasional reversals in direction, and velocities exceeding 10 miles per day are not uncommon (Bumpus, 1969).

In the laboratory the veliger of *N. obsoletus* persists a minimum of 10 days at 25° C and 21 days at 17.5° C (Scheltema, 1967), after which it may settle to the bottom. If conditions are unsuitable the veliger is followed by a pre-adult "creeping-swimming" stage which can postpone settlement many days in cold water (Scheltema, 1965) or where the substrate is unsuitable (Scheltema, 1961). Experimentally the total larval period may reach at least 53 days if sediment is withheld (Scheltema, personal communication). A larva remaining 53 days in the water column with a modest net longshore movement of 5 miles per day will travel 265 miles (423 km) before settling. Taking these figures conservatively as maximum estimates of dispersal per generation, a larva has the capability of travelling along 2/5 of the transect before it metamorphoses.

We infer from these figures that each local population is recruited from larvae of widely mixed provenance, and that extensive gene flow is largely responsible for the prevention of the formation of purely locally adapted gene complexes. Whether gene flow is sufficient to suppress all local genetic differentiation and to maintain complete regional panmixia is problematical and cannot be answered with the data at hand.

Greater insight may be provided if mechanisms of balancing selection and gene flow are viewed in concert rather than in isolation. Extensive gene flow would promote the selection of genes that interact harmoniously with many kinds of genetic background (Mayr, 1963), *i.e.*, they are "good mixers." The partial homogeneity of genetic background conferred by the spread of such genes would favor the diffusion of the same balancing mechanisms throughout the species. These mechanisms would not confront strictly locally adapted gene complexes in which they might have no adaptive value. Once established, balancing mechanisms would further dampen the centripetal tendencies of local adaptation.

Viewed in this way, gene flow and balanced polymorphisms may interact to retard or prevent the genetic "balkanization" of species. The natural history of *N. obsoletus* suggests that for this species gene flow has played the greater role.

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Summary

Six gene loci were characterized by polyacrylamide gel electrophoresis of 5 protein systems in the mud snail, *Nassarius obsoletus*. Snail populations collected at 11 sites along an Atlantic coast transect from Cape Cod, Massachusetts to Beaufort, North Carolina were surveyed for genetic variability at these loci.

Four loci from malate dehydrogenase, tetrazolium oxidase, and leucine aminopeptidase systems are monomorphic for the same alleles througout the transect. Two loci from lactate dehydrogenase (Lc-1) and general protein ((Gp-1) systems are polymorphic in all populations.

The Lc-1 locus has 2 common and 1 rare codominant alleles and the Gp-1 locus segregates for 2 codominant alleles. Alleles of each locus segregate independently of those of the other. Chi-square homogeneity tests indicate homogeneity of allele and genotype frequency throughout the transect at both loci. All populations conform to Hardy-Weinberg equilibrium values, as do pooled populations without indication of Wahlund's effect. Populations partitioned into size (and hence age) classes are also homogeneous in allele frequency.

The remarkable geographic homogeneity of allele frequency may be due to (1) mechanisms of balanced polymorphism that are insensitive to local variations in environment, or (2) extensive gene flow. The potentialities of widespread pelagic dispersal in *N. obsoletus* argue strongly for gene flow; however the 2 agents of gene stablization are mutually complementary and may have acted in concert throughout populations.

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